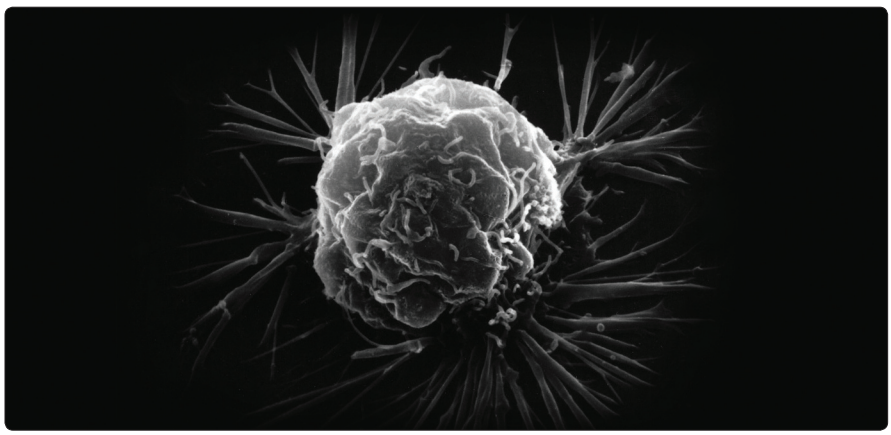
Tech News

Promise and pitfalls of the cancer biomarker search

In the middle of the last decade, the search for cancer biomarkers shifted from a type of gold rush to an elusive quest. The potential payoff is substantial: if oncologists had molecules that could help them find and treat a cancer at an early stage, they expect that they could boost survival. In addition, biomarkers could profile tumors, helping doctors and patients make informed decisions about treatment strategies.

Initially, as the human genome was published, analyzing samples using mass spectrometry looked like a potential gold mine for biomarker discovery. But even after the publication of a number of papers that described biomarker candidates, no new cancer biomarkers have entered the clinic. About five years ago, researchers realized that mining the proteome for cancer biomarkers was much more complicated than they'd anticipated. But improvements in methodology—along with increasing sensitivity in mass spectrometry—are renewing optimism that proteomics can still help uncover new cancer biomarkers.



SEM image of a breast cancer cell. Biomarkers have the promise of identifying the presence of cancer with a simple blood test. Photo courtesy of the National Cancer Institute.

A naive start

Cancer biomarker research is founded on the theory that as a solid tumor grows, it will shed unique proteins into surrounding tissues and into the bloodstream of a patient, says Michael Gillette, a research fellow at the Broad Institute in Cambridge, MA. A decade ago, researchers were optimistic that surface-enhanced laser desorption/ionization (SELDI) and other mass spectrometry–based approaches could quickly distinguish between the serum protein profiles of healthy patients and those with cancer, and use the resulting patterns in diagnostics.

"There turned out to be an enormous number of problems with doing this: everything from the technology to bad experimental design to bad biostatistics and informatics," says Daniel Liebler, professor of biochemistry and director of proteomics at Vanderbilt University School of Medicine in Nashville, TN. But from those missteps also came an important lesson, he says: researchers needed to distinguish between the platforms that discover new biomarkers and the ones that do targeted analysis. "It was just a mismatch of the technology to the objective."

Tools to mine the proteome

Even with the challenges of searching for biomarkers, researchers and funding agencies are still pursuing the promise of these techniques. The National Cancer Institute's Clinical Proteomic Technology Assessment for Cancer (CPTAC) network is one such multidisciplinary collaboration that is refining methods and looking for new cancer biomarkers.

It turns out that proteins that are likely to be biomarkers are also likely to be present in low concentrations relative to the host of other proteins within the blood. Protein concentrations within blood cover a range of up to 12 orders of magnitude, and common proteins such as albumin will dwarf rare proteins. Even if abundant common proteins are removed from samples, undiscovered cancer biomarkers likely lurk among these uncommon proteins at concentrations of no more than 1 ng/mL. And those concentrations hover near the limits of detection for even the most sensitive mass spectrometers.

One of the problems that plagued earlier biomarker studies was the variability and lack of reproducibility of the data. Because of the large number of peptides within a single sample, a single run of a complex mixture within a mass spectrometer does not capture all of the peptides, says Jan Schnitzer, director of the Proteogenomics Research Institute for Systems Medicine in San Diego, CA. Capturing the full complexity of a single sample can require several runs. In addition, biomarker studies have contained unanticipated biases, due to how tissue samples were handled or how the study populations were selected, says Richard Smith of Pacific Northwest National Laboratory in Richland, WA. But researchers are making progress in this area. Liebler and his CPTAC colleagues have done several studies to help standardize proteomics methodologies. Such studies have compared samples that have been stored using different methods and assessed the sources of variability looking for ways to standardize MS-MS measurements across different laboratories.

Researchers are also using a number of tools to dampen some of the biological noise to enable mass spectrometry analysis. Instead of attempting to do shotgun discovery in serum—one of the most complicated mixtures within the body—some groups have moved their

Daniel Liebler, director of the Jim Ayers Institute for Precancer Detection and Diagnosis and professor at Vanderbilt University School of Medicine in Nashville, TN. Courtesy of Dan Liebler.

initial discovery work to proximal fluids relevant to a cancer of interest, such as cyst fluid for ovarian cancer or urine for bladder cancer. The idea is that if we could get closer to the location of the tumor, Gillette says, we might be able to enrich populations of proteins that are ultimately diluted and circulating in the bloodstream. As promising candidates emerge from those samples, researchers can then see if they can be detected in blood.

Although the SELDI approach to biomarker discovery had all sorts of design flaws, it allowed researchers to survey a large number of samples. "That's powerful because people vary, and cancers vary even within a specific subtype," Gillette adds. With current approaches, tremendous resources are required to analyze a single sample and catalogue its inventory of proteins. Realistically, Gillette adds, that means that even large projects might only probe tens of patient samples rather than hundreds or thousands, which creates a small but deep discovery pool. In characterizing a sample containing up to 7000 proteins, they might find 2000 that differ between a cancer population and a control population, but many of these are likely to be false positives because of the small sample size.

Bridging the validation chasm

Even after using selection criteria to prioritize only the most promising early biomarker candidates, researchers might have hundreds—if not thousands—of possibilities. The question then becomes how to move them forward. "That's been the biggest chasm in biomarker development in the past," Gillette says. Antibodies provide one of the most sensitive ways to detect these proteins, but relatively few exist and the development of new ones is both time-consuming and expensive. So Gillette and his colleagues have tried to bridge that chasm with another approach.

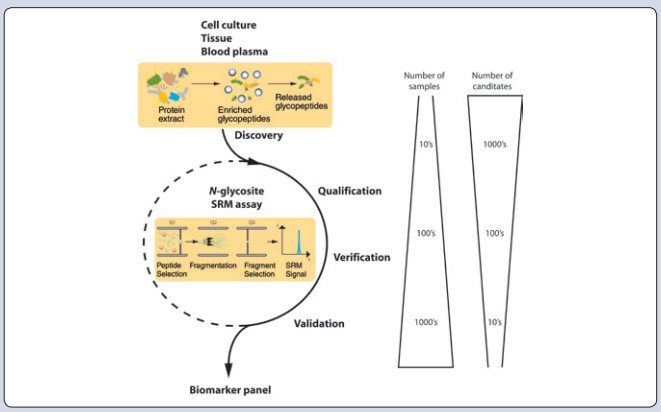
Their solution has been a mass spectrometry–based process called multiple reaction monitoring (MRM) or selective reaction monitoring (SRM). In looking at this subset of biomarker candidates, researchers look for peptide fragments that might be present in the mass spectrometer and reflect the biology of the protein. In MRM, heavily labeled isomers of up to 6 of these peptides for each protein are synthesized with the idea of finding 2–3 peptides that serve as benchmarks that do not interfere with other peptides in plasma. Unlike an antibody-based approach, the mass spectrometry strategy allows for high-throughput screening: researchers can analyze hundreds of peptides at a time.

The strategy offers the opportunity to theoretically quantify nearly any protein without developing new reagents, says

Zeroing in on glycosylated proteins

Ruedi Aebersold and his colleagues at Swiss Federal Institute of Technology in Zürich are narrowing their biomarker search by zeroing in on N-glycosylated proteins (1). These post-translational modifications protect proteins from degradation, giving them a longer half-life in the blood stream, says Ralph Schiess, an associate scientist within the group. And he notes that nearly 80 percent of the known biomarkers are glycosylated.

To find potential N-glycosylated biomarkers, Schiess, Aebersold and their colleagues isolate glycosylated proteins from samples by oxidizing the proteins and then coupling them to a hydrazide resin. Initially, they examine proximal fluids in mouse models. As they find proteins of interest, they'll look for those proteins within mouse serum, followed by human serum. Schiess and his colleagues validate their N-glycosylated biomarker candidates using MRM. The approach has enabled the Aebersold group to uncover four potential prostate cancer biomarkers. Through their biotech company, ProteoMediX (www.proteomedix.ch), they will develop antibodies for these candidates. The researchers are using the same process to search for N-glycosylated biomarkers of ovarian, colon, and pancreatic cancers.



The Aebersold group's strategy for investigating possible cancer biomarkers. Discovery starts with solid phase enrichment of samples, looking for proteins that are glycosylated in cells, tissue and blood. The researchers then use selected reaction monitoring (SRM) assays to look for these protein signatures in human patients. In their prostate cancer studies, they have narrowed their panel to four biomarker candidates and a spinoff company is developing antibodies for those proteins. Reprinted with permission Elsevier © 2009.

Michael Gillette, research fellow at the Broad Institute in Cambridge, MA. An active pulmonary critical care physician, he is also an instructor at the Dana-Farber Cancer Institute and at Harvard Medical School. Photo credit: Maria Nemchuk, Broad Institute.

Eleftherios Diamandis, professor and head of clinical biochemistry at Mount Sinai Hospital in Toronto, Canada. But it does suffer in sensitivity. "It's at least 1000 times less sensitive than the best ELISA," he says.

Emerging strategies are combining MRM with antibodies to create a type of "sandwich" assay. In one version, researchers use an antibody to capture a protein of interest, then digest the protein and use MRM to detect the peptides, Liebler says. Another version, Stable Isotope Standards and Capture by Anti-Peptide Antibodies (SISCAPA), involves digesting the protein first and using antibodies to capture the peptides of interest before using MRM.

The road ahead

It has taken years to develop and implement these new discovery and target analysis strategies to search for new biomarkers. "This is one of those instances where we've been flying the plane and building it at the same time," Gillette says. In the next few years, scientists need to take the technology platforms that they have now developed for discovery and targeted analysis and apply them to relevant test cases. Then, they'll be able to look for early indicators that distinguish a tumor that's responsive to a particular treatment from one that's resistant, Liebler says. "We need to do the gold standard studies that really ask the question: can we systematically apply the pipeline to identify markers for the purposes that we need?"

Many researchers suspect that the undiscovered biomarkers are not likely to be individual silver bullets that clearly indicate cancer, but rather a collection of several different markers. "Cancer is probably the biggest challenge if you talk in terms of early detection," Smith says. "But with the kinds of developments that are taking place, one has to be optimistic that if biomarkers exist, there's going to be success in the coming years."

References

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